Data Supplement

Angiotensin Deficient FVB/N Mice are Normotensive

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Supplemental Data

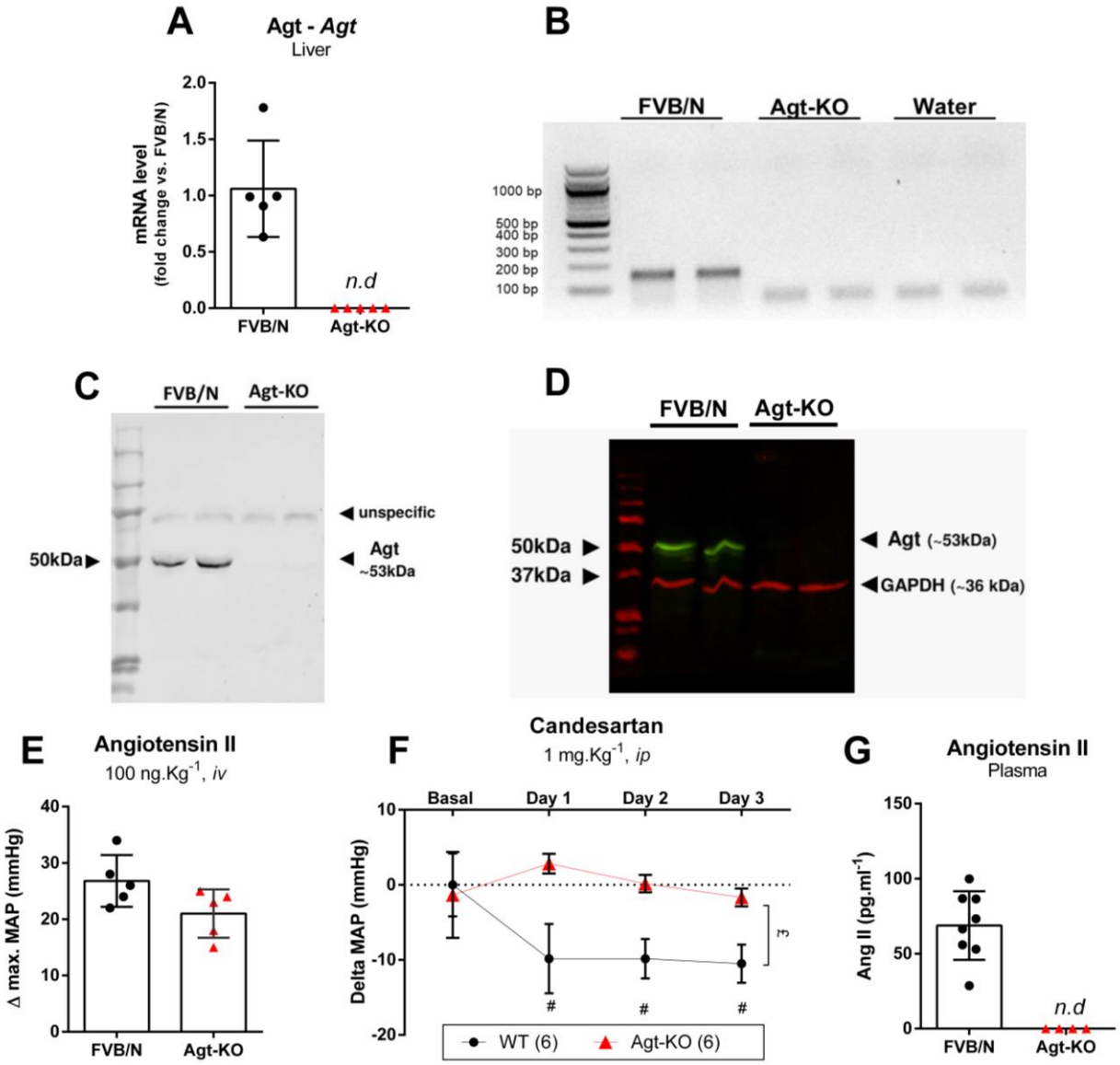

Figure S1 - Validation of FVB/N Agt-KO. Liver *Agt* mRNA levels quantification using RT-qPCR (**A**). Image of an agarose gel containing PCR products from **A** after RT-qPCR. Note, the presence of the Agt expected amplicon ~150 bp exclusively in control (FVB/N) samples (**B**). Absence of the ~53kDa Agt band in plasma (**C)** and liver of Agt-KO using western blots (**D,** green). In **D** the same membrane was incubated with an antibody against the housekeeping protein GAPDH (Red). Maximal mean arterial pressure (MAP) response to a bolus injection of Ang II in freely moving mice (**E**)**.** Baseline MAP response upon 3 intraperitoneal (once a day) injections of candesartan. Baseline MAP was measured in freely moving mice ~22 hours after each injection of candesartan for ~1 hour (**F**). Quantification of plasma Ang II (**G**). For **A, E** and **G** values are mean ± SD. For **F** values are mean ± SD $^{\#}P<0.05$ FVB/N post-candesartan vs FVB/N baseline. $^{£}P<0.05$ Agt-KO vs FVB/N post-candesartan; (2-way ANOVA with repeated measurements followed by Dunnett´s multiple comparison post hoc test); *n.d* = not detected.

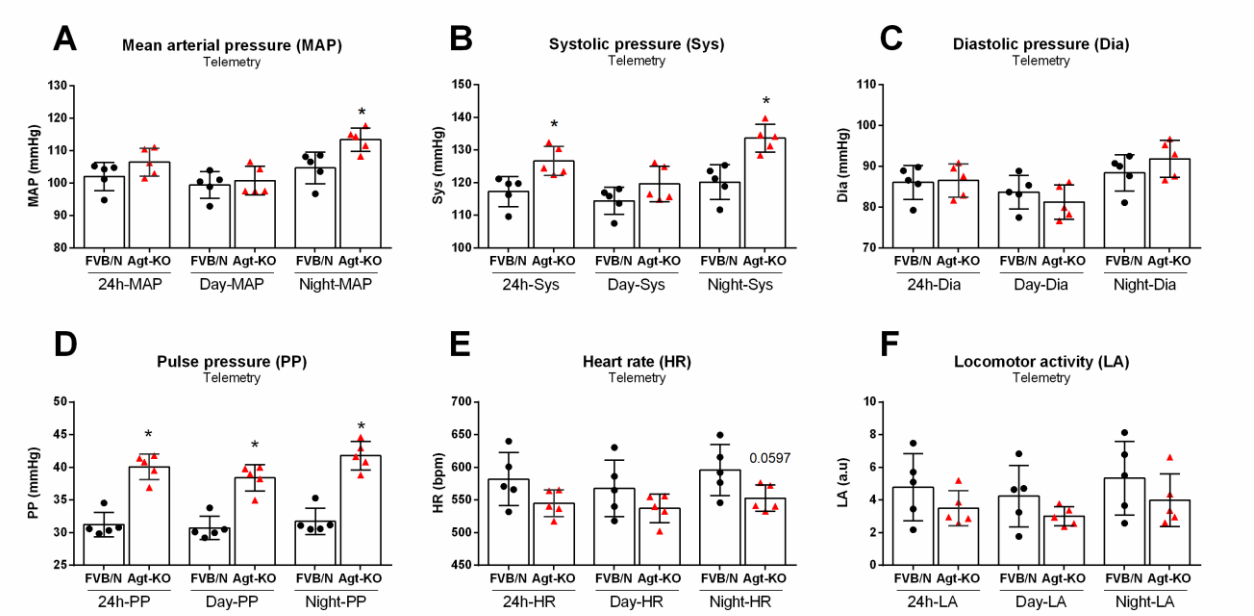

Figure S2 - Differential (24h, day and night) analyses of the telemetric measurements. Mean arterial pressure (**A**), systolic pressure (**B**), diastolic pressure (**C**), pulse pressure (**D**), heart rate (**E**) and locomotor activity (**F**). Pulse pressure was calculated as (systolic - diastolic). Values are mean ± SD **P*<0.05 vs FVB/N (Student's *t* test).

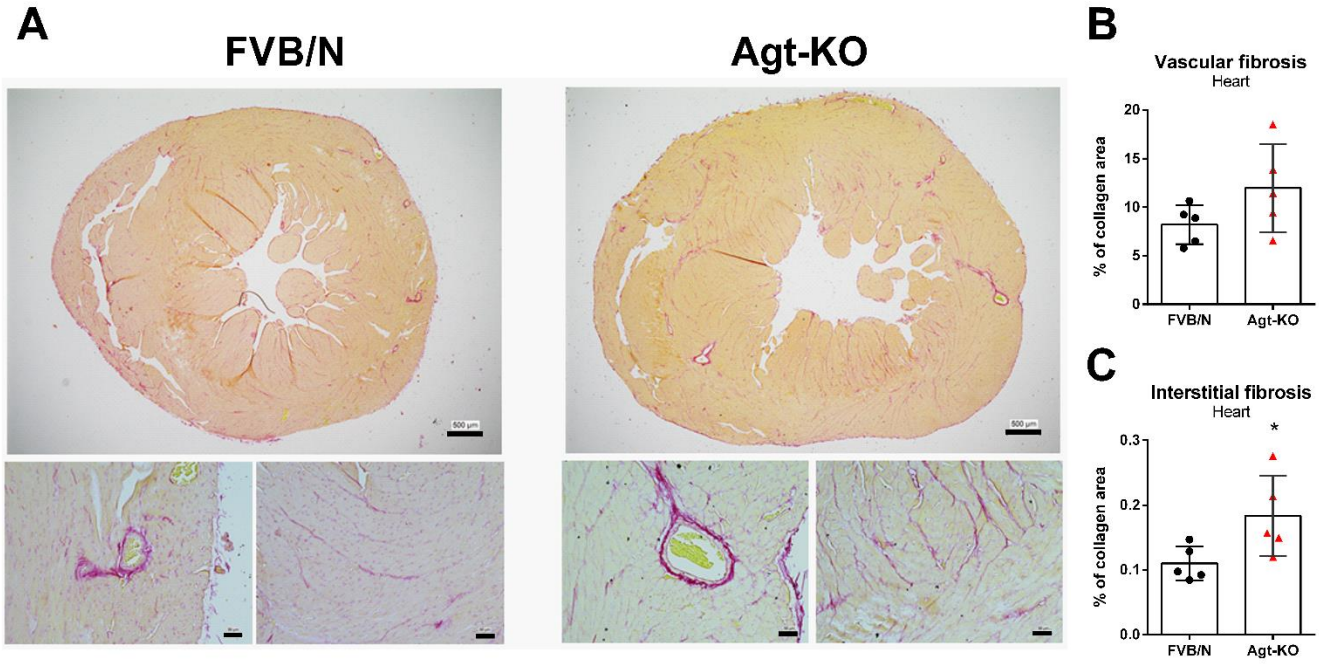

Figure S3 – Ventricular Fibrosis. Histochemical collagen staining with picrosirius-red in representative transverse heart sections (**A**). Quantification of the vascular (perivascular) fibrotic area (**B**). Quantification of the left-ventricular interstitial fibrotic area (**C**). Top panels scale bars are 500 µm, lower panels bars are 50 µm. For **B** and **C** values are mean ± SD **P*<0.05 vs FVB/N (Student's *t* test).

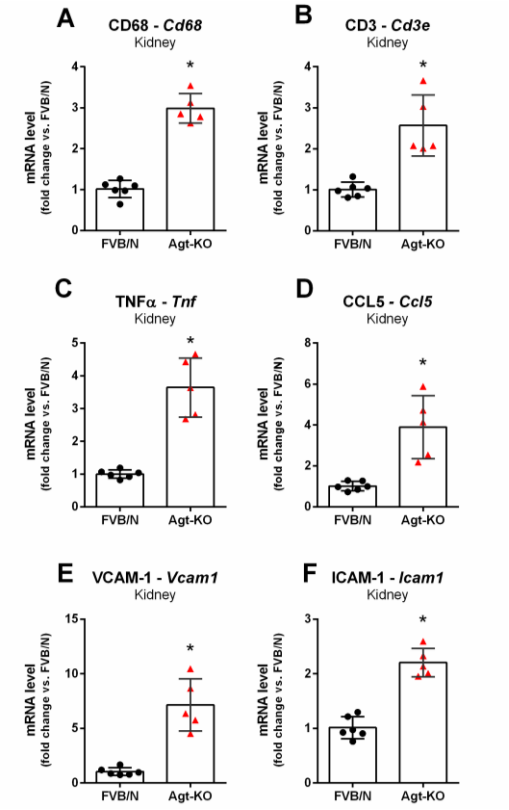

Figure S4 – Renal leucocyte recruitment and inflammation markers. mRNA expression of the macrophage marker, CD68 (**A**), and the T-cell marker, CD3 (**B**). Renal mRNA levels of the cytokines TNFα (**B**) and CCL5 (**C),** and the adhesion molecules VCAM-1 (**E**) and ICAM-1 (**F**). Values are mean ± SD *P<0.05 (Student's t test).

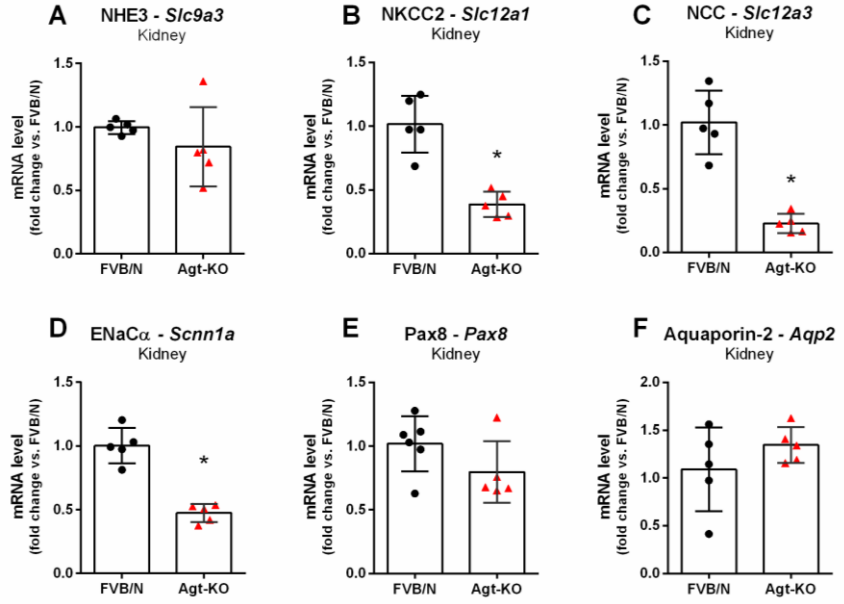

Figure S5 – Kidney tubular protein gene expression. mRNA levels of genes encoding renal sodium transporters: NHE3 (hydrogen exchanger) (**A**), NKCC2 (sodium-potassium-chloride cotransporters) (**B**), sodium-chloride cotransporter (NCC) (**C**), and the α-subunit of the epithelial sodium channel (ENaC-α) (**D**). Renal mRNA levels of the tubular transcription factor Pax8 (**E**) and aquaporin-2 (**F**). Values are mean ± SD *P<0.05 vs FVB/N (Student's t test).

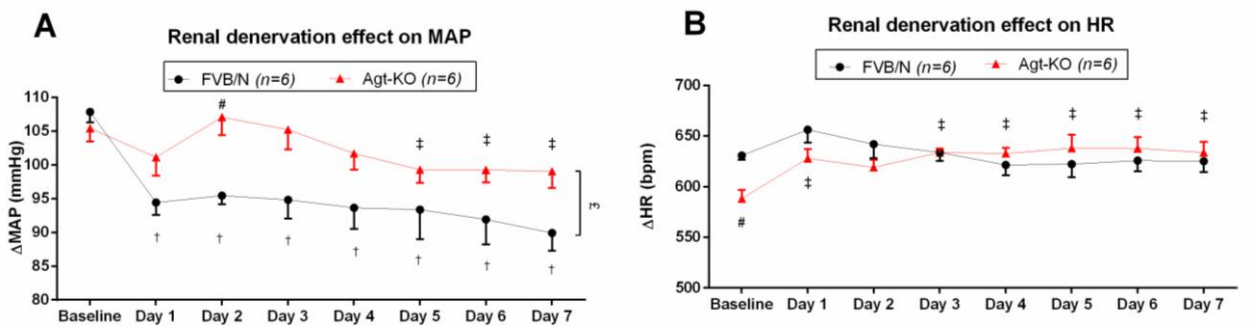

Figure S6 - Effect of renal denervation on cardiovascular homeostasis. **A**, Renal denervation effect on MAP (absolute values). **B**, Renal denervation effect on HR (absolute values). Values are mean ± SE $^{\#}P<0.05$ vs FVB/N at the same time point. $^{\dagger}P<0.05$ FVB/N post-denervation vs FVB/N baseline. $^{\ddagger}P<0.05$ Agt-KO post-denervation vs Agt-KO baseline; $^{£}P<0.05$, Agt-KO vs FVB/N post-denervation; (2-way ANOVA with repeated measurements followed by Bonferroni's multiple comparison post hoc test). MAP = mean arterial pressure, HR = heart rate.

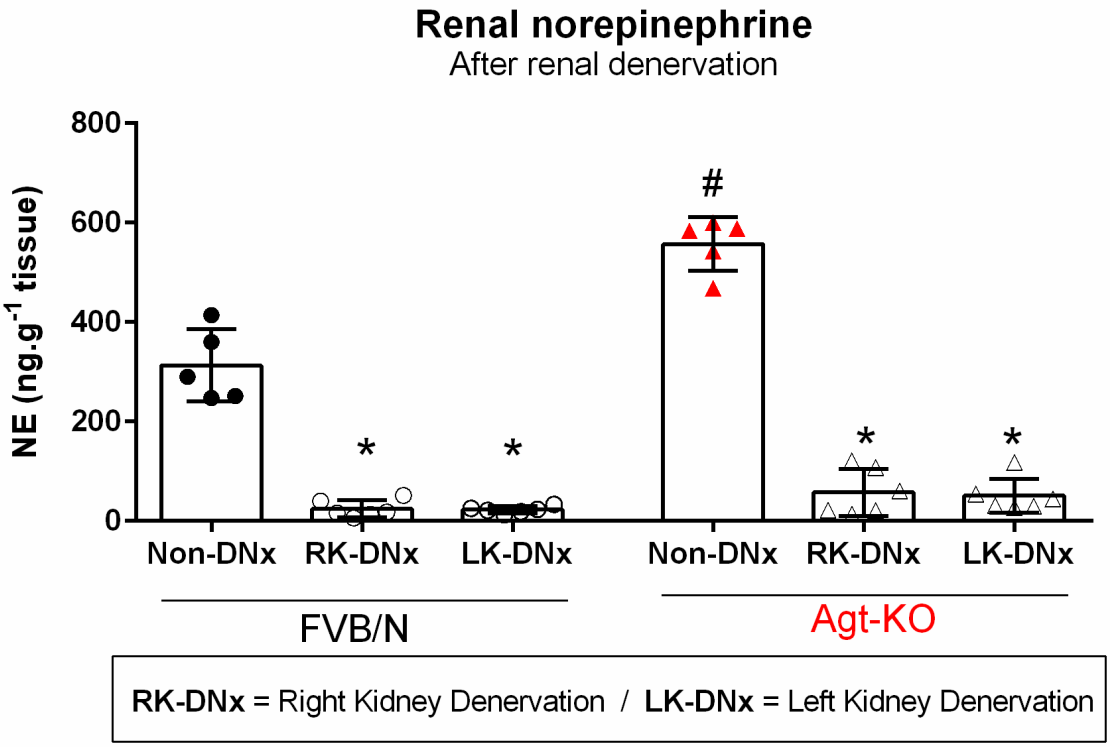

Figure S7 – Validation of bilateral renal denervation. Quantification of renal noradrenaline (NE) to validate the success of the renal denervation procedure (DNx) in each kidney. *$P<0.05$ vs FVB/N or Agt-KO Non-DNx, $^{\#}P<0.05$ vs FVB/N Non-DNx (ANOVA followed by Tukey's multiple comparison test).

Table S1 – Baseline blood pressure in previously published studies with Agt-KO.

	n	Strain	Method	Anesthesia	Age (weeks)	Systolic	Diastolic	MAP	Ref.
Wildtype	14	CBA x C57BL/6	tail-cuff	-	5-6	100.4 ± 4.4	66.8 ± 4.2	77.9 ± 5.3	
Agt-KO	8	CBA x C57BL/6	tail-cuff	-	5-6	66.9 ± 4.1	57.1 ± 1.9	52.5 ± 1.9	Tanimoto et al., 1994
Delta	-	-	-	-	-	**-33.5**	**-9.7**	**-25.4**	
Wildtype	4	CBA x C57BL/6	tail-cuff	-	8	103 ± 1.5	-	-	
Agt-KO	4	CBA x C57BL/6	tail-cuff	-	8	70 ± 3.6	-	-	Ishida et al., 1998
Delta	-	-	-	-	-	**-33**	-	-	
Wildtype	6	CBA x C57BL/6	tail-cuff	-	10	95 ± 6.3	-	-	
Agt-KO	6	CBA x C57BL/6	tail-cuff	-	10	70 ± 2.8	-	-	Tamura et al., 1998
Delta	-	-	-	-	-	**-25**	-	-	
Wildtype	?	CBA x C57BL/6 x NMRI	Catheter[$]	Yes[‡]	8	-	-	85	
Agt-KO	?	CBA x C57BL/6 x NMRI	Catheter[$]	Yes[‡]	8	-	-	50	Kang et al., 2002
Delta	-	-	-	-	-	-	-	**-35**	
Wildtype	?	CBA x C57BL/6	tail-cuff	-	10	97.1 ± 7.1	-	-	
Agt-KO	?	CBA x C57BL/6	tail-cuff	-	10	69.5 ± 2.8	-	-	Kihara, 1998
Delta	-	-	-	-	-	**-27.6**	-	-	
Wildtype	3	129 x C57BL/6J	tail-cuff	-	14	~119[§]	-	-	
Agt-KO	10	129 x C57BL/6J	tail-cuff	-	14	~99[§]	-	-	Kim et al., 1995
Delta	-	-	-	-	-	**-20**	-	-	
Wildtype	?	129/Ola x C57BL/6	tail-cuff	-	7-8	118	-	-	
Agt-KO	?	129/Ola x C57BL/6	tail-cuff	-	7-8	77	-	-	Niimura et al., 1995
Delta	-	-	-	-	-	**-41**	-	-	
Wildtype	7	129/Ola x C57BL/6	Catheter[#]	-	5	-	-	~105[§]	
Agt-KO	7	129/Ola x C57BL/6	Catheter[#]	-	5	-	-	~72[§]	Okubo et al., 1997
Delta	-	-	-	-	-	-	-	-33	
Wildtype	3	129/Ola x C57BL/6	Catheter[#]	-	5	-	-	105 ± 2	
Agt-KO	3	129/Ola x C57BL/6	Catheter[#]	-	5	-	-	70 ± 3	Okubo et al., 1998
Delta	-	-	-	-	-	-	-	-35	
Wildtype	8	129/Ola x C57BL/6	Catheter[#]	-	5-7	-	-	106 ± 3	
Agt-KO	5	129/Ola x C57BL/6	Catheter[#]	-	5-7	-	-	72 ± 3	Tsuchida et al., 1998
Delta	-	-	-	-	-	-	-	-34	

Wildtype	7	129/Ola x C57BL/6	Catheter[#]	Yes[&]	6-10	-	-	96 ± 5	
Agt-KO	4	129/Ola x C57BL/6	Catheter[#]	Yes[&]	6-10	-	-	45 ± 4	Tsuchida et al., 1998
Delta	-	-	-	-	-	-	-	-51	
Wildtype	14	C57BL/6	Catheter[#]	Yes[†]	30	81 ± 4	-	64 ± 4	
Agt-KO	14	C57BL/6	Catheter[#]	Yes[†]	22	58 ± 3	-	39 ± 1	Chen et al., 2010
Delta	-	-	-	-	-	**-23**	-	**-25**	
Wildtype	6	C57BL/6J	tail-cuff	-	?	112± 5	-	-	
Agt-KO	6	C57BL/6J	tail-cuff	-	?	95± 3	-	-	Sun et al., 2003
Delta	-	-	-	-	-	**-17**	-	-	
Wildtype	6	ICR	tail-cuff	-	10	101 ± 6	-	-	
Agt-KO	6	ICR	tail-cuff	-	10	68 ± 5	-	-	Nakamori et al., 2018
Delta	-	-	-	-	-	**-33**	-	-	
Wildtype	?	FVB/N	tail-cuff	-	12-16	120 ± 5	-	-	
Agt-KO	?	FVB/N	tail-cuff	-	12-16	106 ± 7	-	-	Lochard et al., 2003
Delta	-	-	-	-	-	**-14**	-	-	

[#]Catheter implanted in the carotid artery.

[$]Catheter implanted in the femoral artery.

[‡]Chloral hydrate was used as anesthetic.

[†]Urethane and chloralose mixture was used as anesthetic.

[&]Inactin was used as anesthetic.

[§]Value extracted from a bar graph.

? Not described and/or not clearly informed.

Table S2 - Body and wet organ weight of male mice at the age of 13-14 weeks.

Parameter (unit)	FVB/N, *n*=7	Agt-KO, *n*=7
Body weight (g)	29.6 ± 1.7	29. 4 ± 0.9
Tibia length (mm)	17.4 ± 0.3	17.6 ± 0.3
Body weight/tibia length ($g \cdot mm^{-1}$)	1.69 ± 0.10	1.67 ± 0.04
Kidney weight/tibia length ($mg \cdot mm^{-1}$)	12.7 ± 0.51	12.09 ± 1.00
Adrenal weight/tibia length ($mg \cdot mm^{-1}$)	0.34 ± 0.12	0.32 ± 0.13
Spleen weight/tibia length ($mg \cdot mm^{-1}$)	7.28 ± 0.48	7.90 ± 0.77
Lung weight/tibia length ($mg \cdot mm^{-1}$)	9.19 ± 1.02	8.42 ± 0.66

Kidney weight = left kidney. Adrenal gland weight = both adrenals pooled. Values are mean ± SD.

Table S3 - Cardiac baroreflex sensitivity index calculated from equipressor doses of phenylephrine (1-10 µg/kg, *iv*).

Line, *n*	MAP basal **(mmHg)**	MAP peak **(mmHg)**	ΔMAP **(mmHg)**	HR basal **(bpm)**	HR peak **(bpm)**	ΔHR **(bpm)**	BRS ΔPI·ΔMAP^{-1} **($ms \cdot mmHg^{-1}$)**
FVB/N, 8	107.4 ± 7.3	128.4 ± 8.7	20.6 ± 3.1	704.5 ± 51.5	546.6 ± 64.3	-157.9 ± 41.5	1.24 ± 0.42
Agt-KO, 7	108.6 ± 13.5	129.4 ± 13.0	20.9 ± 3.7	666.3 ± 65.9	542.4 ± 71.7	-123.9 ± 19.1	1.07 ± 0.41

Values are mean ± SD.

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angiotensin type 1A and 1B receptor genes duplicate severe abnormal phenotypes of angiotensinogen nullizygotes. *Journal of Clinical Investigation*, *101*(4), 755–760. https://doi.org/10.1172/JCI1899